IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Engelhardt et al.	
Serial No.:	08/479,997	Group Art Unit: 1634
Filed:	June 7, 1995	Examiner: Scott W. Houtteman
For:	OLIGO- OR POLYNUCLEOTIDES, AND OTHER COMPOSITIONS COMPRISING PHOSPHATE MOIETY LABELED NUCLEOTIDES	

Pelham Manor, NY 10803

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION OF DR. CHERYL H. AGRIS, ATTORNEY AT LAW (IN SUPPORT OF THE WRITTEN DESCRIPTION, ENABLEMENT & NON-OBVIOUSNESS OF THE INVENTION CLAIMED IN U.S. PATENT APPLICATION SERIAL NO. 08/479,997)

I, Cheryl H. Agris, hereby declare as follows:

1. I am a solo practitioner in patent law and intellectual property licensing matters, having been so engaged since 1998. Previous to that from 1992 to 1998, I was a patent attorney at Novo Nordisk of North America in New York City. Prior to my position at Novo Nordisk, I was a law clerk in the Biotechnology Group at the law firm of Pennie & Edmonds, also in New York City. I became a patent agent in 1990. As an attorney registered to practice before the U.S. Patent and Trademark Office, my present work involves the preparation and prosecution of U.S. patent applications in the biotechnology, pharmaceutical and chemical fields. I also oversee the foreign prosecution of patent applications. My present work also involves performing patentability and validity studies, infringement analysis and freedom of operation studies. As an intellectual property attorney, I have prepared licensing, consulting and confidentiality agreements for clients. I have also engaged in the peer review of patent prosecution by third parties. My legal and work experience at Novo Nordisk and Pennie & Edmonds is described in my curriculum vitae (CV) which is attached to my Declaration as Exhibit 1.

2. Before entering the intellectual property field, I was a scientist and researcher from 1979 - 1988. In 1979, I was an undergraduate research fellow at the Argonne National Laboratory in Argonne, Illinois. There, I analyzed bile acids isolated from the bile, urine, or serum from children with cholestatic liver disease using gas chromatography and gas chromatography/mass spectroscopy. Later from 1979 to 1986, I was a predoctoral fellow (graduate student) in the Division of Biophysics, Department of Biochemistry at the Johns Hopkins University (JHU) in Baltimore, Maryland. My thesis advisors were Dr. Paul O. P. Ts'o, Chair, Division of Biophysics and Dr. Paul S. Miller. While conducting my thesis research in Dr. Paul Miller's laboratory, I helped formulate methods for synthesizing nonionic oligonucleotide analogs and oligonucleoside methylphosphonates. As a member of Dr. Miller's group, I also studied the effects of oligonucleoside methylphosphonate sequences on the synthesis of VSV (vesicular stomatitis virus) proteins in cell culture and in vitro. In connection with my doctorate that I earned at JHU in 1986, I wrote a dissertation titled "Effects of Chemically Synthesized Oligodeoxyribonucleoside Methylphosphonates on Vesicular Stomatitis Virus Protein Synthesis and Infection," a copy of a portion of which is attached as Exhibit 2. During my education and training at JHU, I co-authored a dozen papers with Dr. Miller, these papers being listed on my CV (Exhibit 1). While working in Dr. Miller's laboratory for several years, I became very familiar with the synthesis of oligonucleotides, particularly the nonionic oligonucleotide analogs, oligonucleoside methylphosphonates, including the chemistry disclosed in Dr. Miller's 1981 Biochemistry paper [Biochemistry 20(7):1874-1880 (1981)] cited in the last two Office Actions issued in the above-identified patent application, and discussed *infra*. During the years 1986 - 1988, I was a research fellow at the Sloan Kettering Institute in New York City working in Dr. Robert Krug's laboratory. Dr. Krug was a member at that time of the Molecular Biology Program at Sloan Kettering; his research focused on transcriptional and translational control of influenza viral protein synthesis. While there, I investigated the mechanism of the block in the splicing of influenza viral NS1 mRNA to NS2 mRNA in vitro using molecular biological and biochemical approaches.

3. As indicated in my CV (Exhibit 1), my formal education includes three degree programs. In 1979, I received my Bachelor of Arts in chemistry from Goucher

College in Towson, Maryland. In 1986, I received my doctoral degree (Ph.D.) from the Johns Hopkins University, School of Hygiene and Public Health (Department of Biochemistry, Division of Biophysics). In 1992, I received my *Juris Doctor* degree from the Brooklyn Law School in Brooklyn, New York.

4. Among my honors, awards and fellowships as listed on page 2 of my CV (Exhibit 1), I was a Richardson Scholar at Brooklyn Law School from 1988-1992. I was an American Cancer Society Fellow at the Sloan Kettering Institute between 1986-1988. In 1984, I received a Student Research Award from the Delta Omega Honorary Public Health Society. Between 1979 and 1986, I was the recipient of predoctoral training grants, first as an NIH predoctoral trainee (1979-1982) and later as a predoctoral trainee under the Albert Szent-Gyorgyi Foundation (1982-1986). When I graduated from college in May 1979, I received General Honors and Honors in Chemistry, in addition to having received the Louise Kelly Award in Chemistry. Previous to that, I worked in the Undergraduate Research Program (January - May 1979) and in the Summer Graduate Student Program (June - August 1979) at Argonne National Laboratory.

5. My experience in continuing legal education has covered a number of significant areas in patent law. At various patent meetings and conferences, I have made several oral presentations on patent issues, including, among others, issues related to the written description and enablement requirements under 35 U.S.C. §112, first paragraph. These presentations included the following: "Why Deposit Biological Materials?" New York, New Jersey, Connecticut, and Pennsylvania Joint Seminar on Developments in Patent Law, April 2000; "Inventorship" National Association of Patent Practitioners meeting, July 1999; "What to Claim in Biotechnology Patent Applications" National Association of Patent Practitioners meeting, October 1997; and "*In re Deuel*, Obviousness Standard for Biotechnology" BIO '96, June 1996.

6. Related to my efforts in the field of patent law are a number of faculty appointments and several oral presentations. These include: speaker on Intellectual Property Considerations in "Angel Financing: Navigating the Legal & Business Issues" at the Citybar Center for Continuing Legal Education (CLE), The Association of the Bar of the City of New York, November 28, 2000; organizer and instructor

at the National Association of Patent Practitioner's 2000 Short Course on Nuts and Bolts of Patent Prosecution (July 2000); instructor at the Sixth, Eighth, Ninth and Tenth Annual Patent Prosecution Workshops: Advanced Claim Drafting and Amendment Writing (1996, 1998, 1999, 2000); and speaker at the Law Seminars: Biotechnology Key Legal & Business Issues, November 18-19, 1999 in Seattle, Washington. Each of these faculty appointments are listed on the third page of my CV (Exhibit 1). Among my oral presentations are two held last year and one each in 1999, 1998, 1997 and 1996. These presentations are also listed on page 3 of my CV (Exhibit 1).

7. I have also attended the following continuing legal education programs in the intellectual property area. These include in the year 2000, Writing and Using Intellectual Property Opinions (Association of the Bar of the City of New York), and the International Intellectual Property Symposium at the Brooklyn Law School. In 1999, I attended the legal program Preparing Legal Opinions 1999: Intellectual Property Due Diligence in Business Transactions, also with the Association of the Bar of the City of New York. In 1998, I took part in the New York, New Jersey, Connecticut and Pennsylvania Joint Seminar on Developments in Patent Law. In 1996, I attended "The Basics of Licensing and Licensing Law." I also attended two Patent Resources Group courses, "Advanced PCT Practice" and "European Patent Office Practice." In 1993, I attended the Practising Law Institute (PLI) program on Technology Licensing and Litigation. These are listed on the first page of my CV (Exhibit 1).

8. Among my publications are seven legal-related articles and fourteen scientific papers, including the dozen papers with Dr. Paul Miller referenced above in Paragraph 2. All of these publications are listed on pages 3-5 of my CV (Exhibit 1). Also listed are some representative U.S. patents among the approximately 150 U.S. patents in which I have participated in the preparation and/or prosecution. These representative U.S. patents are listed on page 4 of my CV (Exhibit 1).

9. I have been engaged by Enzo Biochem, Inc. as a scientific and legal consultant in order to review portions of the current prosecution of U.S. Patent Application Serial No. 08/479,997 (presently titled "Oligo- or Polynucleotides, and Other Compositions Comprising Phosphate Moiety Labeled Nucleotides") that was

filed on June 7, 1995. I am being compensated by Enzo for this review and for making this Declaration. Included for my review were significant portions of the file wrapper for this application, including the original specification (hereinafter "the '997 specification"), the previously pending claims in this application (454-575), changes to independent claims (454, 482, 511 and 539)[1] to be submitted in a response (Amendment Under 37 C.F.R. §1.116) to the July 18, 2000 Office Action, and the latest composite set of claims (454-567) which will be pending in this application following the submission of the aforementioned Amendment After Final. A copy of the previously pending claims (454-575), the changes to the independent claims (454, 482, 511 and 539), and the latest composite set of claims (454-567) are attached to this Declaration as Exhibits 3, 4 and 5, respectively. I have also reviewed the July 18, 2000 Office Action as well as five other previous Office Actions issued on June 20, 1996, May 13, 1997, January 6, 1998, September 29, 1998 and February 3, 1999. I have also reviewed several papers filed in response to the aforementioned office actions. These papers include Applicants' June 23, 2000 Communication, their June 22, 2000 Second Supplemental Amendment, their June 20, 2000 Supplemental Amendment, their January 4, 2000 Amendment Under 37 C.F.R. §1.115, their January 19, 1999 Supplemental Response, their November 20, 1998 Amendment Under 37 C.F.R. §1.116, their July 6, 1998 Amendment Under 37 C.F.R. §1.115, their November 24, 1997 Amendment Under 37 C.F.R. §1.116, and their December 20, 1996 Amendment In Response To June 20, 1996 Office Action And Request For A Three Month Extension Of Time. I generally agree with the substance of Applicants' remarks and positions as set forth in these aforementioned responses. In particular, I agree with the Declaration of Dr. Dean L. Engelhardt In Support Of Adequate Description and Enablement (hereinafter "the Engelhardt Declaration") that was submitted as Exhibit A to Applicants' November 27, 1997 Amendment Under 37 C.F.R. §1.116 In Response To June 25, 1997 Office Action. A copy of the Engelhardt Declaration is attached to my Declaration as Exhibit 6. I have also reviewed the Examiner Interview Summary Records dated November 3, 1998 and August 24, 2000. I also attended the latter August 24, 2000 interview on behalf of Enzo. Moreover, I have read and reviewed the Guidelines for Examination of

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[1] I understand that several dependent claims have also been amended. The affected dependent claims include 455, 459, 461, 466, 476, 480, 483, 487, 489, 494, 504, 508, 510, 512-531, 533, 535-559, 561 and 563-567. I have also reviewed the amendments to the dependent claims which will also be submitted in Applicants' January 18, 2001 Amendment Under 37 C.F.R. §1.116.

Patent Applications Under the 35 U.S.C. 112, ¶1, "Written Description" Requirement, which were published on January 5, 2001 in the Federal Register, Vol. 66, No. 4, Pages 1099-1111. My remarks below, opinions and conclusions with respect to the written description rejections are rendered in light of the aforementioned January 5, 2001 Guidelines. A copy of the January 5, 2001 Guidelines is attached to my Declaration as Exhibit 7.

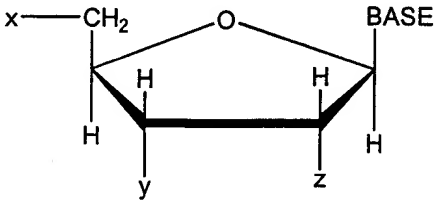
10. As I understand it, the claimed invention as represented by the amended independent claims to be submitted (Exhibit 4) is directed to detectable nucleic acid compositions comprising at least one modified nucleotide.

A. As I understand it, one significant embodiment as set forth in amended claim 454 is an oligo- or polydeoxynucleotide which is complementary to a nucleic acid of interest or a portion thereof. The oligo- or polydeoxynucleotide comprises at least one modified nucleotide having the formula

Sig—PM—SM—BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a base moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof. The PM is attached to SM, the BASE is attached to SM, and Sig is covalently attached to PM directly or through a chemical linkage. The element Sig comprises a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when the modified nucleotide is incorporated into the oligo- or polydeoxynucleotide or when the oligo- or polydeoxynucleotide is hybridized to the complementary nucleic acid of interest or a portion thereof.

B. As set forth in amended claim 482 and as I understand it, another significant embodiment of the claimed invention is an oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof. The oligo- or polydeoxyribonucleotide comprises at least one modified nucleotide having the structural formula:



In the structural formula, BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and is attached to the 1′ position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine. The substituents x, y and z are selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate. Sig is covalently attached directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y, z, and a combination thereof. Sig comprises a non-radioactive label moiety which can be directly or indirectly detected when so attached to the phosphate or when the modified nucleotide is incorporated into the oligo- or polydeoxynucleotide or when the oligo- or polydeoxynucleotide is hybridized to the complementary nucleic acid of interest or a portion thereof.

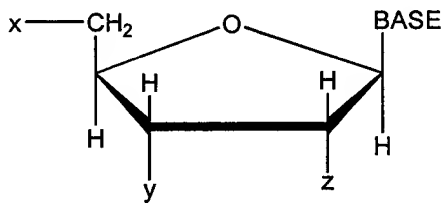
C. I understand that the claimed invention as set forth in amended claim 511 is directed to an oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof. The oligo- or polynucleotide comprises at least one modified nucleotide having the formula

Sig – PM – SM – BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof. PM is attached to SM, BASE is attached to SM, and Sig is covalently attached to PM directly or via a chemical linkage. Sig comprises a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when the modified nucleotide is incorporated into the oligo- or polynucleotide, or when the oligo- or polynucleotide is hybridized to the complementary nucleic acid of interest or a portion thereof. When the oligo- or polynucleotide is an oligoribonucleotide

or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3′ position of a terminal ribonucleotide, the chemical linkage in this oligo- or polynucleotide is not obtained through a 2',3' vicinal oxidation of a 3′ terminal ribonucleotide previously attached to the oligoribonucleotide or polyribonucleotide.

D. I understand another embodiment as set forth in amended claim 539 is an oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof. The oligo- or polynucleotide comprises at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1′ position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine. The elements x, y and z are selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate. Sig is covalently attached directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y and z, and a combination thereof. Sig comprises a non-radioactive label moiety which can be directly or indirectly detected when so attached to the phosphate or when the modified nucleotide is incorporated into the oligo- or polynucleotide, or when the oligo- or polynucleotide is hybridized to the complementary nucleic acid of interest or a portion thereof. Furthermore, when the oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to a terminal PM at the 3′ position of a terminal ribonucleotide, the chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3′ terminal ribonucleotide previously attached to the oligoribonucleotide or polyribonucleotide.

11. I understand that in the latest July 18, 2000 Office Action claims 459-472 and 474-575 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time the application was filed, had possession of the claimed invention. In this rejection, the Examiner indicated that "[s]upport was not found . . ." for the following limitations in claims 459-472 and 474-575:

> Claims 459-463, specific chemical compositions of linkages;
> Claims 464-472, 482-569, specific identity of labels and points of attachment of the "SIG" moiety to internal phosphates;
> Claims 474-477 and 570-575, the "composition" limitation, in addition to the above identified limitations.

12. I understand that in the previous February 3, 1999 Office Action, claims 459-472 and 474-575 were also rejected for "new matter." In that Office Action (pages 2 and 3), the Examiner stated:

> Support was not found where indicated in the specification, nor elsewhere, for the following limitation in Claims 459-472 and 474-575:
> Claims 459-463, specific chemical composition of linkages;
> Claims 464-472, 482-569, specific identity of labels and points of attachment of the "SIG" moiety to internal phosphates;
> Claims 474-477 and 570-575, the "composition" limitation, in addition to the above identified limitations.
> Applicant argues in the response, pages 30-32, that support was found in various portions of the specification. This argument is not persuasive. These portions merely recite support for covalent attachment of a SIG moiety to a phosphate moiety and a SIG moiety to the oxygen on a phosphate moiety. Note, there is no support for the attachment of the SIG moiety to the phosphate atom of the phosphate moiety, only to the oxygen atom.

13. I understand that in the July 18, 2000 Office Action, claims 454-575 were also rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, reasons of record. In that Office Action (pages 3-4), the Examiner indicated:

> Halloran et al., J. Immunol. 96(3):373-378, 1966 (Halloran) discloses the attachment of a specific signal moiety, a protein, to the phosphorus atom of the phosphate moiety using a specific linker, a -C-$(CH_2)_4$-N- chain. In contrast, the claims are drawn to a much broader

> category, a generic "SIG" moiety and linkage, and specific compounds such as those recited in claim 464-magnetic, hormone, metal containing "SIG" moieties, for example.) Thus, the scope of the enablement is not commensurate with the scope of the claims.
>
> In addition, several of the referenced articles are drawn to labeling a mononucleotide and express doubt about labeling an oligonucleotide. Armstrong et al., Eur. J. Biochem. 70:33-38, 1976, teaches that the labeled mononucleotides are "strong competitive inhibitors" of the reaction which is necessary to produce a labeled oligonucleotide from a labeled mononucleotide. See Armstrong, p. 33, col. 1. This reaction is the use of the labeled mononucleotide as a substrate for the polymerase mediated synthesis of the oligonucleotide. While Armstrong teaches that some labeled mononucleotide will be incorporated, Armstrong teaches no guidance as to which of the myriad labels within the scope of these claims will function in the claimed invention. Lacking any guidance in the specification and in view of the breadth of the claims, it would require undue experimentation in order to enable a reasonable number of embodiments of these claims. The skilled artisan would have to experiment with various SIG moieties, linkages and attachment sites, as well as various reaction protocols and protecting groups.

14. I understand that in the previous February 3, 1999 Office Action, claims 454-575 were also rejected for lack of enablement. In that Office Action (pages 3-4), the Examiner stated:

> Applicant argues that the prior art, such as Halloran, supports enablement. This argument is not persuasive for several reasons. Halloran discloses the attachment of a specific signal moiety, a protein, to the phosphorus atom of the phosphate moiety using a specific linker, a -C-$(CH_2)_4$-N- chain. In contrast, the claims are drawn to a much broader category, a generic "SIG" moiety and linkage, and specific compounds such as those recited in claim 464 -- magnetic, hormone, metal containing "SIG" moieties, for example). Thus, the scope of the enablement is not commensurate with the scope of the claims.
>
> In addition, several of the referenced articles are drawn to labeling a mononucleotide and express doubts about labeling an oligonucleotide. Armstrong et al., Eur. J. Biochem. 70:33-38, 1976, teaches that the labeled mononucleotides are "strong competitive inhibitors" of the reaction which is necessary to produce a labeled oligonucleotide from a labeled mononucleotide. See Armstrong, p. 33, col. 1. This reaction is the use of the labeled mononucleotide as a substrate for the polymerase mediated synthesis of the oligonucleotide.
>
> While Armstrong teaches that some labeled mononucleotide will be incorporated, Armstrong teaches no guidance as to which of the myriad labels within the scope of the claims will function in the claimed invention. Lacking any guidance in the specification and in view of the breadth of the claims, it would require undue experimentation in order to enable a reasonable number of

embodiments of these claims. The skilled artisan would have to experiment with various SIG moieties, linkages and attachment sites, as well as various reaction protocols and protecting groups.

15. I further understand that in the July 18, 2000 Office Action, claims 454-575 were rejected under 35 U.S.C. §103 for being unpatentable over Halloran et al., J. Immunol. 96(3):373-378, 1966 or Miller et al., Biochemistry 20(7):1874-1880, 1981, for reasons of record. In the July 18, 2000 Office Action (page 4), the Examiner stated:

> Both Halloran and Miller teach specific labels, (SIG moieties such as proteins and thiophosphates) attached to nucleic acids. See Halloran p. 373, Fig. 1 and col. 2; Miller p. 1874, col. 1. These prior art references differ from the claims in the recitation of some specific labels and linkages. It would have been *prima facie* obvious, however, to one of ordinary skill in the art at the time the invention was made to substitute any linker or label in the methods of Halloran and Miller for the expected benefit of constructing multiple labels. Given the fact that diverse labels such as proteins and thiophosphates, the ordinary artisan would have reasonably expected any moiety used as a label to function in the claimed invention.

On page 5 of the July 18, 2000 Office Action, the Examiner further stated:

> Repeating an important point from a previous Office action, applicants arguments to the 35 U.S.C. §112, first paragraph rejections have provided strong evidence of obviousness and vice-versa. For example, the references used to support enablement, Halloran and Miller add evidence of obviousness.
>
> It is important that the arguments for patentability explain, that the prior art supplied by applicant, for example, Halloran and Miller, can buttress the specification--providing needed evidence that the thin "SIG Phosphate" disclosure both "describes" and "enables" the detailed invention now claimed--but these same prior art references do not render the claims obvious.
>
> Furthermore, the criticisms of the obviousness rejections must be made without undermining the enablement rejection. For example, if arguing that Halloran and Miller are somehow "non-enabled" one must justify how the specification can be enabled. After all, the prior art contains much more detail than that found in the specification.
>
> Finally, the specification is held to a higher standard than the teachings of the prior art supplied to an obviousness rejection. As stated in a previous office action:
>
> 35 U.S.C. §112 provides that, in return for the grant of monopoly, the specification must enable one skilled in the art to "make and use" the invention "without undue experimentation" whereas U.S.C. §103 makes no such requirement. Thus, a teaching of how to use a compound can be entirely adequate to render a claim obvious but, at the same time, entirely inadequate to support the allowance of such a claim.

I also understand that the same rejection for obviousness was made in the previous February 3, 1999 Office Action.

16. As Enzo's consultant and on its behalf, I am making this Declaration in support of the adequate written description, enabling disclosure and non-obviousness of the claims which will be pending after submission of Applicants' Amendment Under 37 C.F.R. §1.116. To the extent that the subject matter is similar with those of the previously pending claims prior to submission of Applicants' Amendment, my remarks are applicable to those previously pending claims as well.

17. Based upon my own training, background and experience, I would submit that at the time this application was first filed in June 1982, a person of ordinary skill in the art relevant to the subject matter being claimed, including nucleic acid modification, synthesis, hybridization and detection, would have possessed or could have been actively pursuing an advanced degree in organic chemistry and/or biochemistry. Such an ordinarily skilled person could also be at least approaching or ranging toward the level of a junior faculty member with 2-5 years of relevant experience, or at least be a postdoctoral student with several years of experience. I consider myself to possess the level of skill and knowledge of at least a person of ordinary skill in the art to which the present application and invention pertains.

Written Description

18. As I understand it, the written description rejection concerns the following three issues:

A. the specific chemical compositions of linkages recited in claims 459-463;

B. the specific identity of labels and points of attachment of the "SIG" moiety to internal phosphates as recited in claims 464-472 and 482-569; and

C. the composition limitations as recited in claims 474-477 and 570-575.

A. **Specific chemical compositions of linkages (claims 459-463)**

19. I understand that claims 459-463 are directed to subject matter where

the chemical linkage comprises a member selected from the group consisting of an olefinic bond at the α-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both (claim 459);

the chemical linkage comprises an allylamine group (claim 460);

the chemical linkage comprises or includes an olefinic bond at the α-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH=CH_2-NH-$$
$$-CH=CH-CH_2-NH-$$
$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-NH-,$$
$$-S-,\quad -\overset{\displaystyle O}{\overset{\|}{C}}-O, \text{ and } -O- \text{ (claim 461);}$$

the chemical linkage of Sig includes a glycosidic linkage moiety (claim 462); and

PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen (claim 463).

20. As a person skilled in the art to which the present invention pertains, I have reviewed the '997 specification as originally filed and it is my opinion and conclusion that the following portions of that disclosure support the above recited chemical linkages:

Chemical Linkage(s)	**Citation In Specification**	**Description in Disclosure**
olefinic bond at the α-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both	Page 11, 2nd ¶ Original Claim 78 Page 11, last ¶ Original Claim 79	that the chemical linkage include an olefinic bond at the α-position relative to B that the chemical linkage group . . . have the structure -CH_2NH-, . . .

Chemical Linkage(s)	Citation In Specification	Description in Disclosure
an allylamine group	Page 11, last ¶ Original Claim 80	Examples of preferred linkages derived from allylamine . . .
olefinic bond at the α-position relative to the point of attachment to the nucleotide or any of the moieties	Page 11, 2nd ¶	preferred that the chemical linkage include an olefinic bond at the α-position relative to B. The presence of such an α-olefinic bond . . .
–CH=CH–NH– –CH=CH–CH$_2$–NH– –CH=CH–CH$_2$–O–CH$_2$–CH(OH)–CH$_2$–NH–	Page 11, line 29 Page 11, l. 29-30	–CH=CH–CH$_2$–NH– –CH=CH–CH$_2$–O–CH$_2$–CH(OH)–CH$_2$–NH–
–S–	Original Claim 82	–S–
–C(=O)–O–	*ibid.*	–C(=O)–O–
–O–	*ibid.*	–O–
glycosidic linkage moiety	Original Claim 25	said Sig chemical moiety is attached by or includes a glycosidic linkage moiety.
PM is a monoP, diP triP and said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oxygen	Page 9, lines 8-14	wherein each of x, y and z represents H–, HO–, HO–P(=O)(OH)–O– . . .
	Page 57, Ex. V	Biotin and polybiotinylated poly-L-lysine were coupled to oligoribonucleotides using a carbodiimide coupling procedure described by Halloran and Parker, J. Immunol., 96 373 (1966).

Thus, it is my opinion and conclusion as a person skilled in the art that the above-cited portions in the disclosure fully support the various chemical linkages recited in the pending claims of this application. The above-cited portions describe such recited chemical linkages in sufficient detail that one skilled in the art can reasonably conclude that the inventors had possession of such chemical linkages.

B. Specific identity of labels and points of attachment of the "SIG" moiety to internal phosphates (claims 464-472 and 482-569)

21. I understand that claims 464-472 and 482-567 (claims 568-569 having been canceled) are directed to subject matter for the following labels of the "SIG" moiety:

Labels (SIG)

Claims 464, 492, 521 and 549 (Listed as (i) through (xvi) below)

(i) biotin

(ii) iminobiotin

(iii) electron dense component

ferritin (claims 465, 493, 522 and 550)

(iv) ligand and a specific ligand binding protein (claims 466, 494, 523 and 551 as amended)

(v) magnetic component

magnetic oxide (claims 467, 495, 524 and 553)

ferric oxide (claims 468, 498, 525 and 552)

(vi) enzyme or an enzyme component

alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase (claims 469, 497, 526 and 554);

(vii) hormone or a hormone component

(viii) metal-containing component

catalytic (claims 470, 498, 527 and 555)

(ix) fluorescent component

fluorescein, rhodamine and dansyl (claims 471, 499, 528 and 556)

(x) chemiluminescent component

(xi) antigen

(xii) hapten

(xiii) antibody or an antibody component

antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an

antigen or hapten (claims 472, 500, 529 and 557);

(xiv) composition comprising the oligo- or polydeoxyribonucleotide . . ., a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed (claims 502, 531 and 559);

polypeptide comprises polylysine (claims 503, 532 and 560);

polypeptide is selected from the group consisting of avidin, streptavidin and anti-hapten immunoglobulin (claims 504, 533 and 561);

(xv) composition of . . ., wherein Sig is a ligand and said polypeptide is an antibody thereto (claims 505, 534 and 562); and

(xvi) Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting (claims 512 and 540).

22. I have reviewed the '997 specification as originally filed and it is my opinion and conclusion as a skilled person in the art that the following portions of that disclosure support the above recited labels or SIG listed as items (i) through (xvi):[2]

Label (Sig)	Citation In Specification	Description in Disclosure
biotin	Page 10, last 2 lines	. . . the preferred A moieties are biotin and iminobiotin.
	Page 97, last ¶, thru Page 98, 1st 4 lines	. . . the chemical moiety A . . . is functionally the equivalent of the Sig component or chemical moiety . . . of this invention.
	Original Claim 92	. . Sig chemical moiety is biotin
iminobiotin	*ibid.*	
electron dense component	Page 97, 1st ¶	The Sig moiety might also include an electron dense component, . . .

[2] **The citations and descriptions listed below are not necessarily intended to be exhaustive of all the support for any given label or Sig. Rather, the citations and descriptions are offered as illustrative support which is non-limiting.**

Label (Sig)	Citation In Specification	Description in Disclosure
ferritin	*ibid.*	such as ferritin, . . .
ligand and a specific binding protein	Page 101, thru Page 102	. . presence of probe is assayed for by the specific binding of the protein to the ligand . . .
magnetic component	Page 97, 1st ¶	magnetic component associated or attached thereto, . . .
magnetic oxide	*ibid.*	such as a magnetic oxide,
ferric oxide	*ibid.*	or magnetic iron oxide, . . .
enzyme or an enzyme component:	Page 96, 2nd ¶	The Sig moiety could comprise an enzyme or enzymic material
alkaline phosphatase	*ibid.*	such alkaline phosphatase,
acid phosphatase	Original Claim 41 Original Claim 197	said enzyme is acid phosphatase group . . . acid phosphatase . . .
β-galactosidase	Page 36, 3rd ¶ Also Original Claim 84	direct enzymes such as . . . or β-galactosidase . . . said enzyme is β-galactosidase.
ribonuclease	Page 96, 2nd ¶	or ribonuclease.
glucose oxidase	*ibid.*	glucose oxidase,
peroxidase	*ibid.*	horseradish peroxidase,
hormone or a hormone component	Page 102, 1st ¶	3. Hormone receptors and other receptors on the surface of the cell . . .
metal-containing component	Original Claim 28	. . metal-containing component
catalytic	Original Claim 83 Original Claim 174	said Sig chemical moiety includes or comprises a catalytic metal component . . . catalytically active metal.
fluorescent component	Page 96, 1st ¶	The Sig moiety could include a fluorescing component
fluorescein, rhodamine or dansyl	*ibid.*	such as fluorescein or rhodamine or dansyl.

Label (Sig)	Citation In Specification	Description in Disclosure
chemiluminescent component	Page 97, 1st ¶	The Sig component or moiety could include . . . a chemiluminescent component
antigen	Original Claim 28 See also Page 88, 2nd ¶	an antigen . . . fix to a solid matrix a specific antigen and bind to this antigen an antibody directed against this antigen which itself has been biotinylated.
hapten	Page 97, 1st ¶	could include a hapten component
antibody or an antibody component	Original Claim 28	or antibody component.
antigen or hapten capable of complexing with antibody . . .	Original Claim 136	said Sig chemical moiety includes an antigenic or hapten component capable of complexing with an antibody specific to said component.
antibody or an antibody component capable of complexing with an antigen or hapten	*ibid.* See also Page 88, last ¶	The use of the antigen-antibody system for detecting either antigen or antibody is well known.
composition . . . oligo- or polynucleotide . . polypeptide and moiety which can be detected	Original Claims 167 & 168	A polynucleotide comprising one or more nucleotides . . . coupled to a polypeptide, . . . having attached . . . one or more Sig chemical moieties
polypeptide . . . polylysine	Original Claim 56	said polypeptide is a polylysine.
polypeptide . . . avidin, streptavidin and anti-hapten immunoglobulin	Page 25, last 2 lines Page 26, 3rd ¶ Page 34, 1st ¶	One polypeptide detector for the biotinyl-type probe is avidin. A preferred probe for biotin-containing nucleotides and derivatives is streptavidin, an avidin-like protein . . . We can haptenize this secondary antibody with biotin and thus generate two anti-hapten IgG populations, . . .

Label (Sig)	Citation In Specification	Description in Disclosure
Sig is a ligand and said polypeptide is an antibody	Page 101, 1st ¶	. . presence of probe is assayed for by the specific binding of the protein to the ligand . . .
self-signaling or self-indicating or self-detecting	Page 82, 1st ¶	Of special importance and significance . . . self-signaling or self-indicating or self-detecting nucleic acids,

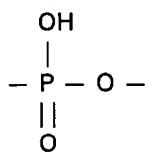
Thus, it is my opinion and conclusion as a person skilled in this art that the above-cited portions in the disclosure fully support the various labels which embody Sig in the pending claims of this application. The above-cited portions which I find to be in general quite explicit describe the claimed labels or Sig in sufficient detail that one skilled in the art can reasonably conclude that the inventors had possession of such claimed labels or Sig.

2. Attachment of SIG to Internal Phosphates

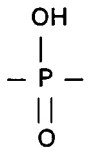
23. I understand that among claims 464-472 and 482-567 (claims 568-569 having been canceled), certain of these are directed to the points of attachment of the "SIG" moiety to internal phosphates:

An oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide having the structural formula . . . wherein x, y and z are selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate and wherein Sig is covalently attached directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y, z, and a combination thereof . . . (claim 482);

oligo- or polydeoxyribonucleotide of . . . wherein said covalent attachment is selected from . . .



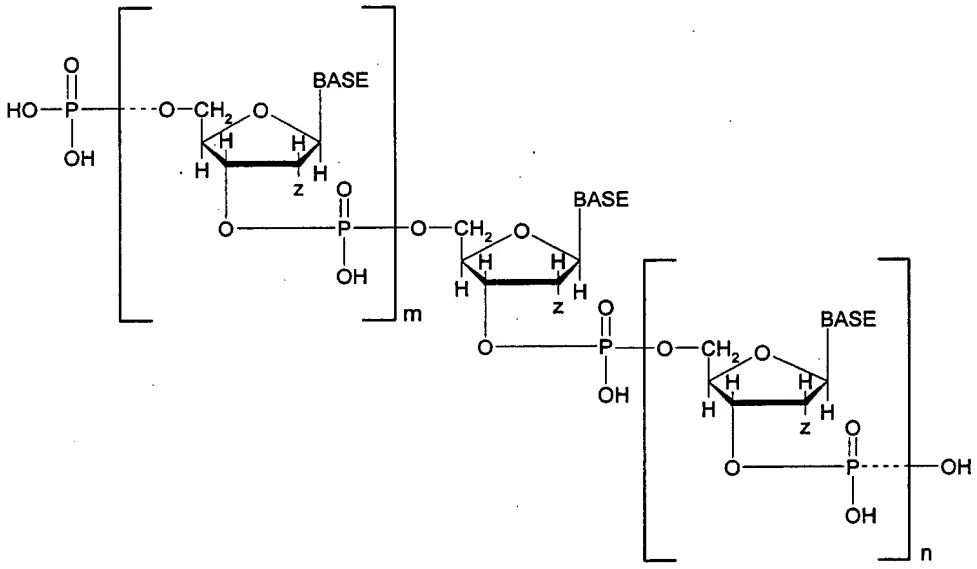
and



(claims 485, 514 and 542)

oligo- or polydeoxyribonucleotide of . . . wherein said x and y each comprise a member selected from the group consisting of mono-, di or tri-phosphate and said Sig moiety is covalently attached to either or both of said x and y through a phosphorus or phosphate oxygen(claim 491, 520 and 548);

oligo- or polydexoyribonucleotide of . . . having the structural formula:

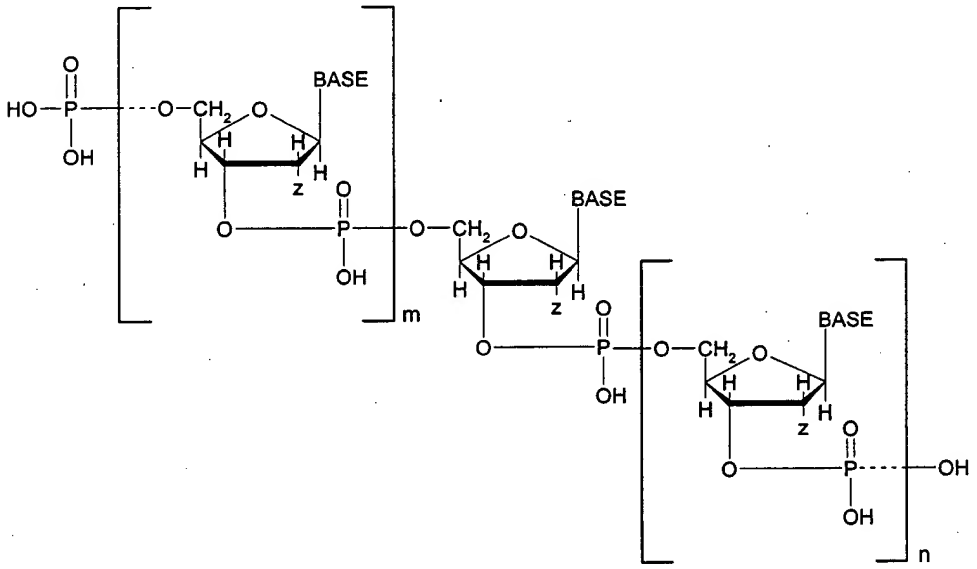


wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula (claim 510);

oligo- or polynucleotide . . . having the structural formula Sig – PM – SM – BASE . . . Sig is covalently attached to PM directly or via a chemical linkage (claim 511) [recited in Paragraph 10C above];

oligo- or polyribonucleotide comprising at least one nucleotide having the structural formula . . . Sig is covalently attached to x, y or z directly or through a chemical linkage (claim 539) [recited in Paragraph 10D above]; and

oligo- or polyribonucleotide of . . ., having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula (claim 567).

24. I have reviewed the '997 specification as originally filed. As a person of ordinary skill in the art, it is my opinion and conclusion that the original disclosure sufficiently describes the attachment of the Sig moiety to the phosphorus atom as to reasonably convey that Applicants had possession of such claimed subject matter at the time the invention was made in June 1982.

25. As a patent practitioner, I have lectured and written on the requirements of 35 U.S.C. §112, including the Written Description requirements under the first paragraph of §112, and I have also submitted comments on the Interim Written Description Guidelines issued July 7, 1998, 63 FR 32,639. I wish to point out that Applicants' disclosure and their claimed subject matter also meets the January 5, 2001 Written Description Guidelines (Exhibit 7), particularly with respect to the attachment of Sig to the phosphorus atom of the phosphate moiety. Those guidelines provide that the written description requirement is met when the patent specification describes the claimed invention in "sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention" (citing *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed. Cir. 1991) 66 FR 1099 (2001). According to these guidelines, possession may be shown by showing that the invention was "ready for patenting" such as by

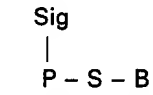
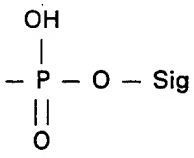
the disclosure of drawings or other descriptions of the invention that are sufficiently specific to enable a person skilled in the art to practice the invention. *Id.* It is well established case law that when the elements recited in the claims are supported by corresponding language in the text, examples, drawings, or other disclosure in the specification, the written description requirement is satisfied and no further analysis is required. *In re Bowen*, 492 F.2d 859 (C.C.P.A. 1974). Furthermore, if new claims are proposed during prosecution, each claim must be expressly, implicitly or inherently supported in the originally filed disclosure and each claim must include all elements which applicant has described as essential. 66 FR at 1105. To establish inherency, it must be clear from any extrinsic evidence provided in the missing descriptive matter is necessarily present in the thing described in the reference and that it would be so recognized by persons of ordinary skill. *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949 (Fed. Cir. 1999).

26. I would also point out that training materials have also been provided in connection with the Revised Interim Written Description Guidelines issued on December 21, 1999. A decision tree was included with those training materials and is attached to my Declaration as Exhibit 8. Although revisions are expected to the training materials in view of the final Written Description Guidelines, these training materials appear to be still in effect. 66 FR at 1099. When a claim of broader scope is added, the question posed is "Is an element(s) missing from the claim?" If the answer is "yes", the question posed is "Is the missing element(s) described by applicant as being an essential or critical feature of the new claim as a whole?" If the answer is "no", the question posed is "Is there express, inherent or implicit support for the claim as a whole?" If the answer is "yes", the written description requirement is met.

27. For reasons explained in the succeeding subsections of this paragraph (A-D), it is my opinion and conclusion that the written description requirement has been met.

A. First, several structures are depicted and descriptions given where "Sig" is attached to the phosphate moiety. As described on pages 8-10 in the Engelhardt Declaration (Exhibit 6), such structures are found variously in the specification, for example, on page 94, last paragraph, and continuing through

page 95, first paragraph:

> Still further, nucleotides in accordance with the practices of this invention include the nucleotides having the formula
>
> 
>
> wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety. In these special nucleotides the P moiety is attached to the 3' and/or the 5' position of the S moiety when the nucleotide is deoxyribonucleotide and at the 2', 3' and/or 5' position when the nucleotide is a ribonucleotide. The base B is either a purine or a pyrimidine and the B moiety is attached from the N1 or the N9 position to the 1' position of the sugar moiety when said B moiety is a pyrimidine or a purine, respectively. The Sig chemical moiety is covalently attached to the phosphoric acid P moiety via the chemical linkage
>
> 
>
> said Sig, when attached to said P moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded or DNA, RNA or DNA-RNA hybrid.

Later in the specification, on page 96, and continuing through the first paragraph on page 98, other descriptions are provided wherein Sig is attached to the phosphate moiety:

> By way of summary, as indicated hereinabove with respect to the make-up of the various special nucleotides in accordance with this invention, the special nucleotides can be described as comprising a phosphoric acid moiety P, a sugar moiety S and a base moiety B, a purine or pyrimidine, which combination of P-S-B is well known with respect to and defines nucleotides, both deoxyribonucleotides and ribonucleotides. The nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig. The chemical moiety Sig so attached to the nucleotide P-S-B is capable of rendering or making the resulting nucleotide, now comprising P-S-B with the Sig moiety attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, especially a double-stranded polynucleotide, such as double-stranded DNA, a double-stranded RNA or a double-stranded DNA-RNA hybrid. The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide in accordance with this invention and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation.

The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an enzyme or enzymic material, such as alkaline phosphatase, glucose oxidase, horseradish peroxidase or ribonuclease. The Sig moiety could also contain a fluorescing component, such as fluorescin or rhodamine or dansyl. If desired, the Sig moiety could include a magnetic component associated or attached thereto, such as a magnetic oxide or magnetic iron oxide, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The Sig moiety might also include an electron dense component, such as ferritin, so as to be available by observation. The Sig moiety could also include a radioactive isotope component, such as radioactive cobalt, making the resulting nucleotide observable by radiation detecting means. The Sig moiety could also include a hapten component or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a polysaccharide or oligosaccharide or monosaccharide, which is capable of complexing with or being attached to a sugar or polysaccharide binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a chemiluminescent component.

As indicated in accordance with the practices of this invention, the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.

The Sig component of the nucleotides in accordance with this invention and the nucleotides and polynucleotides incorporating the nucleotides of this invention containing the Sig component are equivalent to and useful for the same purposes as the nucleotides described in the above-identified U.S. patent application Serial No. 255,223. More specifically, the chemical moiety A described in U.S. patent application Serial No. 255,223 is functionally the equivalent of the Sig component or chemical moiety of the special nucleotides of this invention. Accordingly, the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm as described in U.S. patent application Ser. No. 255,223, as indicated by the dotted line connecting B and A of the nucleotides of U.S. Serial No. 255,223. The various linker arms or linkages indentified in U.S. Ser. No. 255,223 are applicable to and useful in the preparation of the special nucleotides of this invention.

B. Furthermore, as set forth on pages 10-11 of the Engelhardt Declaration (Exhibit 6), there are nine separate instances where Sig is described in the specification as being attached to the phosphate moiety P (as well as the sugar moiety S and/or the base moiety B):

Specification	Description
page 90, last paragraph	. . . and a signalling chemical moiety Sig covalently attached thereto, either to the P, S or B moiety.
page 93, first paragraph	. . . include a chemical moiety Sig covalently attached to the P, S and/or B moieties.
page 96, first paragraph	. . . by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig.
page 98, first paragraph	. . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
page 103, first full paragraph	. . . and the signalling or self-detecting moiety, Sig, covalently attached to either the P, S or B moieties, as indicated hereinabove, . . .
page 104, first paragraph	. . . nucleotides in accordance with this invention containing the above-described components P, S, B and Sig . . .
page 105, first paragraph	. . . the nucleotides of this invention include the P, S, B and Sig components wherein the Sig is covalently attached to either the P, S or B moieties
page 105, second paragraph	The moiety Sig attached to the special nucleotides of this invention containing the other moieties or components P, S, B provide a site per se for the attachment thereto, the Sig component, . . .
page 106, first paragraph	. . . the special P, S, B and Sig-containing nucleotides of this invention, . . .

Upon seeing such structures and their descriptions in the specification, one of ordinary skill in the art would clearly understand that "Sig" may be attached to either the oxygen or phosphorus atom of the phosphate moiety. As described in the Engelhardt Declaration (Exhibit 6), methods are provided in the specification and are well known in the art for attaching a substituent to the phosphorus and to the oxygen atom. Therefore, in my opinion, the written description requirement has been met.

C. Thirdly, I hold the same opinion and I have reached the same conclusion in applying the analysis set forth in the decision tree in the training materials (Exhibit 8) provided in connection with the Written Description Guidelines. As a person of ordinary skill in the art, I also recognize that the scope of claims 459-472 and 474-567 is somewhat broader than the original claims. Specifically, the originally filed claims (see, for example, original claims 141) contained the requirement that "Sig" be attached to the oxygen atom in the phosphate moiety (PM). The currently pending claims do not contain such a requirement. This missing element is not an essential or critical feature of the new claims as a whole because there is no specific requirement set forth in the specification that "Sig" be attached to the oxygen atom in the phosphate moiety.

D. Further, in my opinion and conclusion, there is inherent support for the subject matter of claims 459-474 and 474-567. As a person of ordinary skill in the art, it is my opinion and conclusion that a reading of the specification, including Example V and the extrinsic evidence detailed in the Engelhardt Declaration (Exhibit 6), reasonably conveys that in accordance with the present invention, when a substituent is depicted as being attached to a phosphate moiety (PM), that such a substituent could be attached to either an oxygen or phosphorus atom.

Accordingly, it is my opinion and conclusion that Applicants' claimed subject matter wherein the Sig moiety is attached to the phosphorus or oxygen atom of the phosphate moiety meets the requirements for written description.

C. <u>Composition limitations (claims 474-477 and 570-575)</u>

28. In view of the cancellation of claims 570-575 to be effected by Applicants' Amendment Under 37 C.F.R. §1.116, my remarks below are directed to claims 474-477. These last-mentioned claims contain the following subject matter:

a composition comprising the oligo- or polydeoxyribonucleotide of claim 454, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed (claim 474);

the composition . . . wherein said polypeptide comprises polylysine (claim 475);

the composition . . . wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin (claim 476); and

the composition . . . wherein Sig is a ligand and said polypeptide is an antibody thereto (claim 477).

29. I have reviewed the '997 specification as originally filed and it is my opinion and conclusion as a skilled person in the art that the following portions of that disclosure support the above recited compositions:

Composition(s)	Citation In Specification	Description in Disclosure
comprising oligo- or polydeoxyribonucleotide . . . , a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed	Page 8, last ¶ Page 97, last ¶, thru Page 98, 1st 4 lines	wherein A represents a moiety . . . which is capable of forming a detectable complex with a polypeptide . . . the chemical moiety A described in U.S. patent application Serial No. 255. 223 is functionally the equivalent of the Sig component or chemical moiety . . . of this invention.
wherein said polypeptide comprises polylysine	Original Claim 56	said polypeptide is a polylysine.
polypeptide . . . avidin, streptavidin and anti-hapten immunoglobulin	Page 25, last 2 lines Page 26, 3rd ¶ Page 34, 1st ¶	One polypeptide detector for the biotinyl-type probe is avidin. A preferred probe for biotin-containing nucleotides and derivatives is streptavidin, an avidin-like protein . . . We can haptenize this secondary antibody with biotin and thus generate two anti-hapten IgG populations, . . .
Sig is a ligand and said polypeptide is an antibody	Page 101, 1st ¶	. . presence of probe is assayed for by the specific binding of the protein to the ligand . . .

30. Based upon the above-cited portions, it is my opinion and conclusion as a person skilled in the art that the specification as originally filed fully supports the compositional subject matter in claims 474-477. I find that the disclosure provides sufficient detail that a person skilled in the art can reasonably conclude that the inventors had possession of the subject matter in claims 474-477 at the time the

application was originally filed in June 1982.

Enablement

31. As a person of at least ordinary skill in the art to which the present invention pertains, it is my opinion and conclusion that the original disclosure of the '997 specification was enabling and permitted the practice of the subject matter of claims 454-567 without undue experimentation. My reasons for concluding that the '997 specification is enabling are set forth below.

32. First, although Halloran et al. only teaches one moiety, other moieties are disclosed in the specification and are known in the art. Specifically, Example V of the instant specification discloses a method for attaching biotin, one of the embodiments for Sig, to the phosphate moiety of a mononucleotide and an oligonucleotide that are coupled to a protein, poly-L-lysine. Furthermore, as detailed on pages 11 and 12 in the Engelhardt Declaration (Exhibit 6), the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms in a nucleotidyl phosphate or phosphoric acid moiety were already known in the art at the time the initial application was filed in June 1982.

33. Second, I would like to respond to statements made in the July 18, 2000 Office Action regarding the Armstrong et al. reference. Specifically, it is stated on page 3 in that Office Action that:

> Armstrong et al., Eur. J. Biochem. 70:33-38, 1976, teaches that the labeled mononucleotides are "strong competitive inhibitors" of the reaction which is necessary to produce a labeled oligonucleotide from a labeled mononucleotide. See Armstrong, p. 33, col. 1. This reaction is the use of the labeled mononucleotide as a substrate for the polymerase mediated synthesis of the oligonucleotide.

The conclusion is reached in the Office Action that:

> . . . Lacking any guidance in the specification and in view of the breadth of the claims, it would require undue experimentation in order to enable a reasonable number of embodiments of these claims. The skilled artisan would have to experiment with various SIG moieties, linkages and attachment sites, as well as various reaction protocols and protecting groups.

In response, it is my opinion and conclusion as a person skilled in the art that the labeled mononucleotides referenced in Armstrong et al. were strong competitive inhibitors because unmodified nucleoside triphosphates (NTPs) were present in the assay mixture. This would not be the case or a factor in the present invention. Armstrong et al. actually show that it is possible to incorporate such modified mononucleotides and that modified NTPs may be used as substrates. Although Armstrong et al. does disclose that some modified nucleotides are better than others in terms of binding to RNA polymerase, a person skilled in the art would expect that some routine testing or refinement is necessary.

Non-Obviousness

34. As a person of at least ordinary skill in the art to which the present invention pertains, it is my opinion and conclusion that the claimed subject matter at hand in the form of claims 454-567 would not have been obvious at the time the invention was made from a reading of either Halloran et al. [J. Immunol. 96(3):373-378 (1966)] or Miller et al. [Biochemistry 20(7):1874-1880 (1981)]. My reasons for the non-obviousness of the invention of claims 454-567 are set forth in the paragraphs below.

35. It is asserted in the last Office Actions that both Halloran et al. and Miller et al. teach specific labels. Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute any linker or label in the methods of Halloran and Miller for the expected benefit of constructing multiple labels. In paragraph 11 of the February 3, 1999 Office Action, it is requested that arguments for patentability explain that the prior art supplied by applicant can buttress the specification providing needed evidence that the "SIG-phosphate" disclosure both "describes" and "enables" the detailed invention now claimed, but that these same prior art references do not render the claims obvious.

36. As one skilled in the art, it is my opinion and conclusion that neither Halloran et al. nor Miller et al. actually disclose or suggest a "Sig-phosphate" moiety as defined in the Serial No. 08/479,997 specification. Specifically, Halloran et al. is

directed to covalently conjugating polynucleotides to proteins as a means of stimulating antibody formation. Miller et al. is directed to non-ionic oligonucleotide analogs. These analogs may be more easily taken up by cells than oligodeoxyribonucleotides and are resistant to cleavage by a variety of nucleases. Neither of these modified polynucleotides were ever made for labeling purposes, nor were such use of Halloran's or Miller's modified polynucleotides ever suggested by the cited documents. Certainly, neither the protein in Halloran et al. nor the methyl group in Miller et al. would be considered a detectable label as defined in the art or a detectable Sig chemical moiety as defined in the '997 specification. Before the advent of the invention now claimed, there had been no suggestion regarding attaching a Sig group, that is, a moiety capable of non-radioactive detection when attached to the phosphate moiety of at least one nucleotide in an oligo- or polydeoxyribonucleotide or polynucleotide.

37. As one skilled in the art to which this invention pertains, I wish to point out that there had been teachings regarding modifying the phosphate moiety with various substituents, but none of these substituents comprised a Sig-phosphate as set forth in the present claims. Once such a concept for modifying the phosphate moiety of a nucleotide was formulated, one of ordinary skill in the art would have looked to prior art references in order to develop methods for obtaining the oligo- or poly(deoxyribo)nucleotides of the present invention. As a specific example, Halloran et al. disclosed methods for coupling proteins to nucleotides and oligonucleotides. This reference may be used as a general reference for coupling amino acid moieties to the phosphate moiety. There is no disclosure in Halloran et al., however, that a final oligo- or poly(deoxyribo)nucleotide comprising at least one modified nucleotide of the formula set forth in the pending claims could be obtained.

38. With respect to the cited Miller reference, I am very familiar with the chemistry employed to make oligonucleoside methylphosphonates, having been a graduate student in Dr. Paul Miller's laboratory during the years 1979-1986. During this tim , I and other members of Dr. Miller's group looked to procedures published on obtaining oligonucleotides in order to synthesize oligonucleoside methylphosphonates. For example, the condensing agent used in the cited Miller et al. reference, mesitylene sulfonyl tetrazolide, was used in synthesizing

oligonucleotides. A copy of Stawinski et al. [Nucleic Acids Research 4:353-371 (1977)] which describe the use of mesitylene sulfonyl tetrazolide in synthesizing oligonucleotides is attached as Exhibit 9. Also attached is section II.A. of my Ph.D. thesis (Exhibit 2) detailing strategies used in synthesizing oligonucleoside methylphosphonates. Although the thesis was not submitted until January 1986, the synthetic work described on pages 13-16 actually took place between 1979-1983. I was personally involved in synthesizing oligonucleoside methylphosphonates in June 1982. As described in these pages, methods known in the art for synthesizing oligodeoxyribonucleotides were used as a basis for synthesizing oligonucleoside methylphosphonates. However, neither I nor in my opinion, others of ordinary skill in the art had a reasonable expectation of success that these procedures could be successfully used in preparing oligonucleoside methyl phosphonates. For example, Stawinski et al. (Exhibit 9) merely provided a starting point regarding possible reaction conditions that could be used. There is no suggestion in Stawinski et al., however, that arylsulfonyltetrazoles could or even should be used to synthesize oligonucleoside methylphosphonates. The references cited were primarily used as guidelines for trying to formulate methods for synthesizing oligonucleoside methylphosphonates.

39. Similarly, it appears that the Miller and Halloran references were used as guidelines in formulating the oligo- or poly(deoxyribo)nucleotides of the present invention. The disclosures in each of these references would be sufficiently enabling for one of ordinary skill in the art for formulating procedures for synthesizing the oligo- or poly(deoxy)ribonucleotides of the present invention. There was no suggestion, however, that these procedures could or should be used to obtain, nor would one of ordinary skill in the art have a reasonable expectation of success in obtaining the oligo- or poly(deoxy)ribonucleotides of the present invention. Thus, it is my opinion and conclusion that a person of ordinary skill in the art would not have arrived at the invention claimed in U.S. Patent Serial No. 08/479,995 from a reading of the cited Miller or Halloran references, or even by combining the two references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1/17/01
Date

Cheryl H. Agris
Cheryl H. Agris, Ph.D.
Attorney At Law

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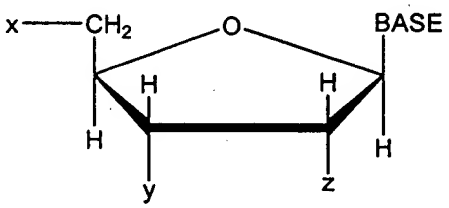
454. (Amended) An oligo- or polydeoxynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxynucleotide comprising at least one modified nucleotide having the formula

Sig—PM—SM—BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a base moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or through a chemical linkage, said Sig [being a moiety capable non-radioactive detection] comprising a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when said modified nucleotide is incorporated into said oligo- or polydeoxynucleotide or when said oligo- or polydeoxynucleotid is hybridized to said complementary nucleic acid of interest or a portion thereof.

482. (Amended) An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein z is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate; and

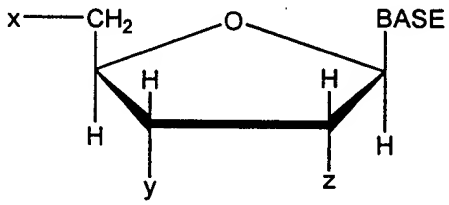
wherein Sig is covalently attached [to x, y or z] directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y, z, and a combination thereof, said Sig [being a moiety capable of non-radioactive detection] comprising a non-radioactive label moiety which can be directly or indirectly detected when so attached to [x, y or z] said phosphate or when said modified nucleotide is incorporated into said oligo- or polydeoxynucleotide or when said oligo- or polydeoxynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

511. (Amended) An oligo- or [polyribonucleotide] polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one [ribonucleotide] modified nucleotide having the formula

Sig – PM – SM – BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM [at a position of SM selected from the 2', 3' and 5' positions, or combinations thereof], said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig [being a moiety capable of non-radioactive detection] comprising a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when said modified nucleotide is incorporated into said oligo- or [polyribonucleotide] polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not [a cleaved 3' terminal ribonucleotide] obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said [oligo- or polyribonucleotide] oligoribonucleotide or polyribonucleotide.

539. (Amended) An oligo- or [polyribonucleotide] polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein z is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate; and

wherein Sig is covalently attached [to x, y or z] directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y and z, and a combination thereof, said Sig [being a moiety capable of non-radioactive detection] comprising a non-radioactive label moiety which can be directly or indirectly detected when so attached to [x, y or z] said phosphate or when said modified nucleotide is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of inter st or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to [y of] a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not [a cleaved 3' terminal ribonucleotide] obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said [oligo- or polyribonucleotide] oligoribonucleotide or polyribonucleotide.

454. An oligo- or polydeoxynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxynucleotide comprising at least one modified nucleotide having the formula

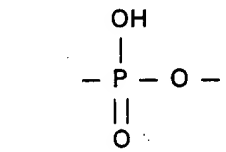
Sig—PM—SM—BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a base moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or through a chemical linkage, said Sig comprising a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when said modified nucleotide is incorporated into said oligo- or polydeoxynucleotide or when said oligo- or polydeoxynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

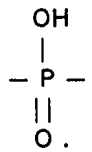
455. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig is or renders the nucleotide or the oligo- or polydeoxyribonucleotide self-signaling or self-indicating or self-detecting.

456. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety comprises at least three carbon atoms.

457. The oligo- or polydeoxyribonucleotide of claim 454, wherein said covalent attachment is selected from the group consisting of



and



458. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

459. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the α-position relative to the point of attachment to the nucleotide, a $-CH_2NH-$ moiety, or both.

460. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises an allylamine group.

461. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises or includes an olefinic bond at the α-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH=CH_2-NH-$$

$$-CH=CH-CH_2-NH-$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH,}{\underset{|}{CH}}-CH_2-NH-,$$

$$-S-,\quad -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and }-O-.$$

462. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

463. The oligo- or polydeoxyribonucleotide of claim 454, wherein said PM is monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oyxgen.

464. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

465. The oligo- or polydeoxyribonucleotide of claim 464, wherein said electron dense component comprises ferritin.

466. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig is select d from the group consisting of a ligand and a specific ligand binding protein.

467. The oligo- or polydeoxyribonucleotide of claim 464, wherein said magnetic component comprises magnetic oxide.

468. The oligo- or polydeoxyribonucleotide of claim 467, wherein said magnetic oxide comprises ferric oxide.

469. The oligo- or polydeoxyribonucleotide of claim 464, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

470. The oligo- or polydeoxyribonucleotide of claim 464, wherein said metal-containing component is catalytic.

471. The oligo- or polydeoxyribonucleotide of claim 464, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

472. The oligo- or polydeoxyribonucleotide of claim 464, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

473. The oligo- or polydeoxyribonucleotide of claim 454, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

474. A composition comprising the oligo- or polydeoxyribonucleotide of claim 454, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

475. The composition of claim 474, wherein said polypeptide comprises polylysine.

476. The composition of claim 474, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-hapten immunoglobulin.

477. The composition of claim 474, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

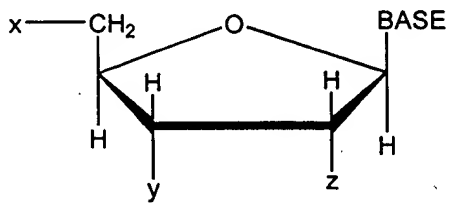
478. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

479. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at the 2' position thereof.

480. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has oxygen atoms at each of the 2' and 3' positions thereof.

481. The oligo- or polydeoxyribonucleotide of claim 454, comprising at least one ribonucleotide.

482. An oligo- or polydeoxyribonucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

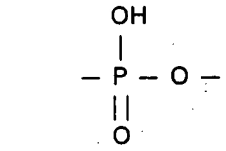
wherein z is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate; and

wherein Sig is covalently attached directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y, z, and a combination thereof, said Sig comprising a non-radioactive label moiety which can be directly or indirectly detected when so attached to said phosphate or when said modified nucleotide is incorporated into said oligo- or polydeoxynucleotide or when said oligo- or polydeoxynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof.

483. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig is or renders the nucleotide or the oligo- or polydeoxyribonucleotde self-signaling or self-indicating or self-detecting.

484. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety comprises at least three carbon atoms.

485. The oligo- or polydeoxyribonucleotide of claim 482, wherein said covalent attachment is selected from the group consisting of



and

486. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

487. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the α-position relative to the point of attachment to the nucleotide, a $-CH_2NH-$ moiety, or both.

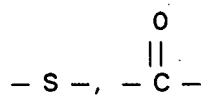
488. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises an allylamine group.

489. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises or includes an olefinic bond at the α-position relative to the point of attachment to x, y or z, or any of the moieties:

$$-CH=CH_2-NH-$$

$$-CH=CH-CH_2-NH-$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-NH-,$$



490. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

491. The oligo- or polydeoxyribonucleotide of claim 482, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and said Sig moiety is covalently attached to either or both of said x and y a phosphorus atom or phosphate oyxgen.

492. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

493. The oligo- or polydeoxyribonucleotide of claim 492, wherein said electron dense component comprises ferritin.

494. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig is selected from the group consisting of a ligand and a specific ligand binding protein.

495. The oligo- or polydeoxyribonucleotide of claim 492, wherein said magnetic component comprises magnetic oxide.

496. The oligo- or polydeoxyribonucleotide of claim 495, wherein said magnetic oxide comprises ferric oxide.

497. The oligo- or polydeoxyribonucleotide of claim 492, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

498. The oligo- or polydeoxyribonucleotide of claim 492, wherein said metal-containing component is catalytic.

499. The oligo- or polydeoxyribonucleotide of claim 492, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

500. The oligo- or polydeoxyribonucleotide of claim 492, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

501. The oligo- or polydeoxyribonucleotide of claim 482, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

502. A composition comprising the oligo- or polydeoxyribonucleotide of claim 482, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

503. The composition of claim 500, wherein said polypeptide comprises polylysine.

504. The composition of claim 502, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-hapten immunoglobulin.

505. The composition of claim 502, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

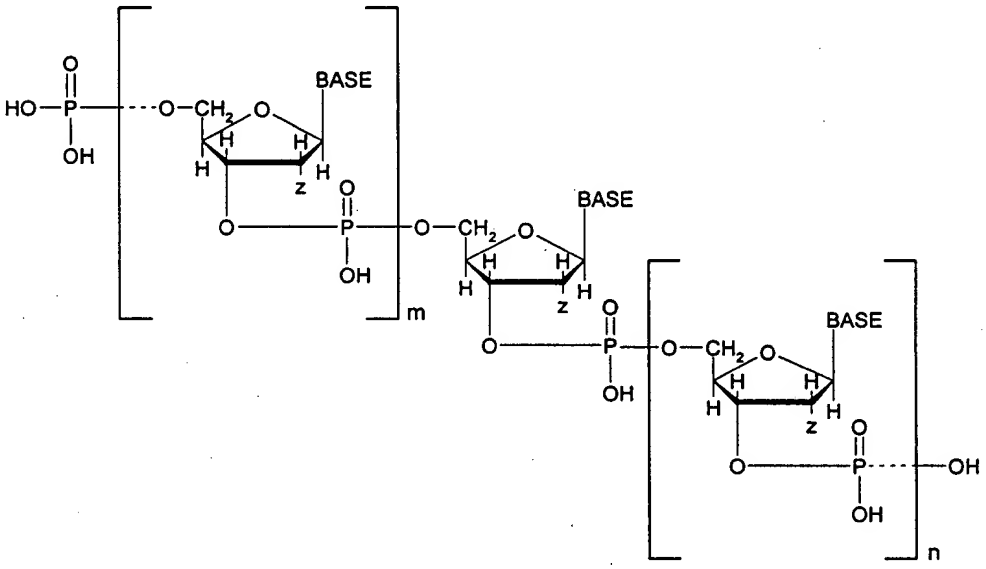
506. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

507. The oligo- or polydeoxyribonucleotide of claim 506, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

508. The oligo- or polydeoxyribonucleotide of claim 506, wherein both y and z of said terminal nucleotide comprise an oxygen atom at each of the 3' and 2' positions thereof, respectively.

509. The oligo- or polydeoxyribonucleotide of claim 482, comprising at least one ribonucleotide.

510. The oligo- or polydexoyribonucleotide of claim 482, having the structural formula:



, wherein m and n represent integers from 0 up to about 100,000, and wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

511. An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide having the formula

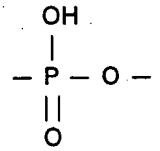
Sig – PM – SM – BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig comprising a non-radioactive label moiety which can be directly or indirectly detected when attached to PM or when said modified nucleotide is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide, and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

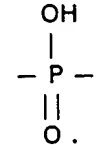
512. The oligo- or polynucleotide of claim 511, wherein said Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting.

513. The oligo- or polynucleotide of claim 511, wherein said Sig moiety comprises at least three carbon atoms.

514. The oligo- or polynucleotide of claim 511, wherein said covalent attachment is selected from the group consisting of



and



515. The oligo- or polynucleotide of claim 511, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

516. The oligo- or polynucleotide of claim 511, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the α-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both.

517. The oligo- or polynucleotide of claim 511, wherein said chemical linkage comprises an allylamine group.

518. The oligo- or polynucleotide of claim 511, wherein said chemical linkage comprises or includes an olefinic bond at the α-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH=CH_2-NH-$$

$$-CH=CH-CH_2-NH-$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-NH-,$$

$$-S-,\quad -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and }-O-.$$

519. The oligo- or polynucleotide of claim 511, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

520. The oligo- or polynucleotide of claim 511, wherein said PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or a phosphate oxygen.

521. The oligo- or polynucleotide of claim 511, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

522. The oligo- or polynucleotide of claim 521, wherein said electron dense component comprises ferritin.

523. The oligo- or polynucleotide of claim 511, wherein Sig is selected from the group consisting of a ligand and a specific ligand binding protein.

524. The oligo- or polynucleotide of claim 521, wherein said magnetic component comprises magnetic oxide.

525. The oligo- or polynucleotide of claim 524, wherein said magnetic oxide comprises ferric oxide.

526. The oligo- or polynucleotide of claim 521, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

527. The oligo- or polynucleotide of claim 521, wherein said metal-containing component is catalytic.

528. The oligo- or polynucleotide of claim 521, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

529. The oligo- or polynucleotide of claim 521, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

530. The oligo- or polynucleotide of claim 511, wherein said oligo- or polynucleotide is terminally ligated or attached to a polypeptide.

531. A composition comprising the oligo- or polynucleotide of claim 511, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

532. The composition of claim 531, wherein said polypeptide comprises polylysine.

533. The composition of claim 531, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-hapten immunoglobulin.

534. The composition of claim 531, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

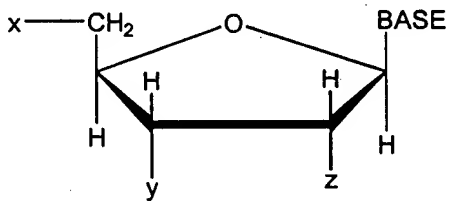
535. The oligo- or polynucleotide of claim 511, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polynucleotide.

536. The oligo- or polynucleotide of claim 535, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at the 2' position thereof.

537. The oligo- or polynucleotide of claim 535, wherein the sugar moiety of said terminal nucleotide has an oxygen atom at each of the 2' and 3' positions thereof.

538. The oligo- or polynucleotide of claim 511, comprising at least one deoxyribonucleotide.

539. An oligo- or polynucleotide which is complementary to a nucleic acid of interest or a portion thereof, said oligo- or polynucleotide comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

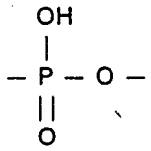
wherein z is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate; and

wherein Sig is covalently attached directly or through a chemical linkage to at least one phosphate selected from the group consisting of x, y and z, and a combination thereof, said Sig comprising a non-radioactive label moiety which can be directly or indirectly detected when so attached to said phosphate or when said modified nucleotide is incorporated into said oligo- or polynucleotide, or when said oligo- or polynucleotide is hybridized to said complementary nucleic acid of interest or a portion thereof, provided that when said oligo- or polynucleotide is an oligoribonucleotide or a polyribonucleotide and when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not obtained through a 2',3' vicinal oxidation of a 3' terminal ribonucleotide previously attached to said oligoribonucleotide or polyribonucleotide.

540. The oligo- or polynucleotide of claim 539, wherein said Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting.

541. The oligo- or polynucleotide of claim 539, wherein said Sig moiety comprises at least three carbon atoms.

542. The oligo- or polynucleotide of claim 539, wherein said covalent attachment is selected from the group consisting of



and

543. The oligo- or polynucleotide of claim 539, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

544. The oligo- or polynucleotide of claim 539, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the α-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both.

545. The oligo- or polynucleotide of claim 539, wherein said chemical linkage comprises an allylamine group.

546. The oligo- or polynucleotide of claim 539, wherein said chemical linkage comprises or includes an olefinic bond at the α-position relative to x, y or z, or any of the moieties:

$$-CH=CH_2-NH-$$

$$-CH=CH-CH_2-NH-$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-NH-,$$

$$-S-,\quad -\overset{\displaystyle O}{\overset{||}{C}}-O,\text{ and }-O-.$$

547. The oligo- or polynucleotide of claim 539, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

548. The oligo- or polynucleotide of claim 539, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and Sig moiety is covalently attached to either or both of said x and y a phosphorus atom or a phosphate oxygen.

549. The oligo- or polynucleotide of claim 539, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

550. The oligo- or polynucleotide of claim 549, wherein said electron dense component comprises ferritin.

551. The oligo- or polynucleotide of claim 539, wherein Sig is selected from the group consisting of a ligand and a specific ligand binding protein.

552. The oligo- or polynucleotide of claim 549, wherein said magnetic component comprises magnetic oxide.

553. The oligo- or polynucleotide of claim 552, wherein said magnetic oxide comprises ferric oxide.

554. The oligo- or polynucleotide of claim 549, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

555. The oligo- or polynucleotide of claim 549, wherein said metal-containing component is catalytic.

556. The oligo- or polynucleotide of claim 549, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

557. The oligo- or polynucleotide of claim 549, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

558. The oligo- or polynucleotide of claim 539, wherein said oligo- or polynucleotide is terminally ligated or attached to a polypeptide.

559. A composition comprising the oligo- or polynucleotide of claim 539, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

560. The composition of claim 559, wherein said polypeptide comprises polylysine.

561. The composition of claim 559, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-hapten immunoglobulin.

562. Th composition of claim 559, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

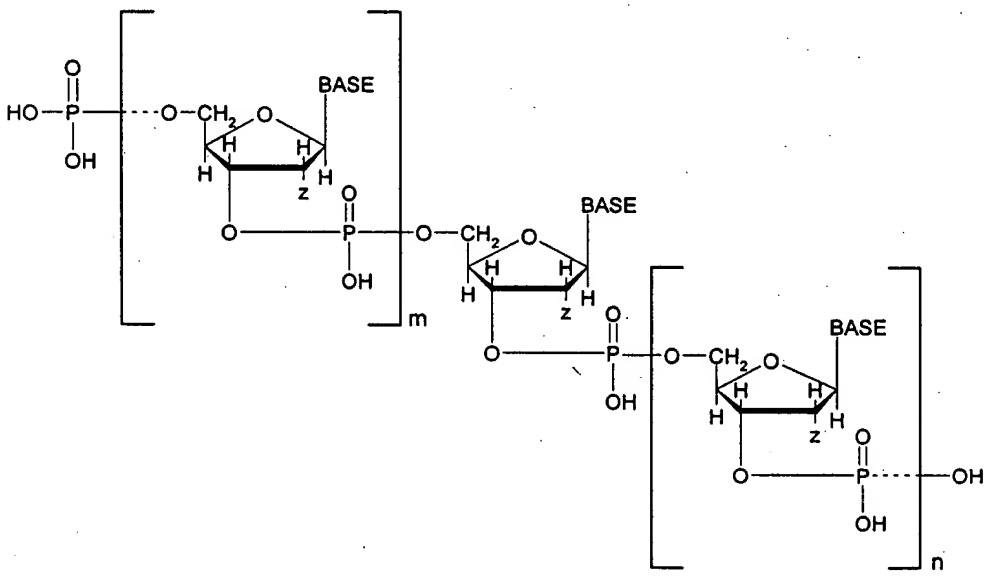
563. The oligo- or polynucleotide of claim 539, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polynucleotide.

564. The oligo- or polynucleotide of claim 563, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

565. The oligo- or polynucleotide of claim 563, wherein both y and z of said terminal nucleotide comprise an oxygen atom at each of the 3' and 2' positions thereof, respectively.

566. The oligo- or polynucleotide of claim 539, comprising at least one deoxyribonucleotide.

567. The oligo- or polynucleotide of claim 539, having the structural formula:



, wherein m and n represent integers from 0 up to about 100,000, and wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

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